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Compartmentalization drives the evolution of symbiotic cooperation

Running head: Compartmentalization drives symbiotic cooperation

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Summary:

Across the tree of life, hosts have evolved mechanisms to control and mediate interactions with symbiotic partners. We suggest that physical structures to spatially separate symbionts, termed compartmentalization, is a common mechanism used by hosts. Compartmentalization allows host to: (i) spatially separate symbionts and control their reproduction; (ii) reward cooperative symbionts and punish or stop interactions with non-cooperative symbionts; and (iii) reduce direct conflict among different symbionts strains in a single host. Compartmentalization has allowed hosts to increase the benefits that they obtain from symbiotic partners across a diversity of interactions, including legumes and rhizobia, plants and fungi, squid and *Vibrio*, insects and nutrient provisioning bacteria, plants and insects, and the human microbiome. In cases where compartmentalization has not evolved, we ask why not. We argue that when partners interact in a competitive hierarchy, or when hosts engage in partnerships which are less costly, compartmentalization is less likely to evolve. We conclude that compartmentalization is key to understanding the evolution of symbiotic cooperation.

Key words:

Compartmentalization, symbiosis, cooperation, punishment, mutualism

1. Introduction

Across the tree of life, hosts have evolved key adaptations to control and mediate interactions with symbiotic partners. In the vast majority of these partnerships, a host is interacting simultaneously with multiple – potentially competing – symbionts [1-4]. Simultaneously interacting with multiple partners can be beneficial, especially if there is variation in the benefits conferred [5]. Multiple partners can help to buffer against variable environments and changing conditions [6]. However, it can also entail costs, with conflicts among symbionts being potentially harmful to the host [4,7-12]. Specifically, interacting with multiple partners creates a potential tragedy of the commons where less beneficial (i.e. called cheaters if they benefit from non-reciprocating and are evolutionarily derived from mutualists; see refs. 13-14) potentially share in the collective benefits the host provides, while paying lower costs. A major question in symbiosis research is how such conflicts are avoided.

Vertical transmission, where symbionts are passed on from one generation to the next, can help align the fitness interests of hosts and symbionts, by increasing relatedness among symbionts sharing a host [11,15-16]. Empirical research has demonstrated how vertical transmission effectively limits symbiont diversity, reduces conflicts, and can drive higher levels of dependency [17-19]. However, symbiotic partnerships involving multiple, unrelated and horizontally-transmitted partner species are likewise pervasive [1,20-21], raising the question of how conflict among competing symbionts [8] is avoided when relatedness among symbionts is low, and symbionts are transmitted horizontally.

A common mechanism for helping to control symbionts is compartmentalization, where physical structures are used to separate microbes in space. These structures allow hosts to stabilise cooperation in ways that would be impossible in the absence of such structures [22]. Here we argue that compartmentalization can help stabilise cooperation by allowing hosts to: (i) spatially separate symbionts and control their reproduction; (ii) reward cooperative symbionts and punish or stop interactions with non-cooperative symbionts; and (iii) reduce direct conflict among different symbionts strains in a single host. We highlight the diverse functions that compartmentalization serves and illustrate its ubiquity. Finally, we ask: how can multi-partner mutualisms be explained where compartmentalization has not evolved?

2. Compartmentalization as a mean to spatially separate symbionts and control their reproduction

Containing the growth and spread of microbes to specific structures can help hosts to harness their benefits, and prevent microbial parasitism [23-27]. Many, but not all, endosymbiotic partnerships have parasitic ancestry - and by recruiting symbionts from the environment, hosts face heightened risk of infections [28-33]. Host control is essential to ensure that microbes do not invade all host tissues. In evolving the ability to restrict a microbe to particular tissues, hosts are able to limit direct negative effects, and gain benefits of partnerships. Many of these cases represent strict forms of compartmentalization (**Table 1**). By strict compartmentalization, we mean that symbionts – often as a single genotype – are fully enveloped by well-defined, physical compartments. By physically isolating symbionts in this way, hosts can regulate symbiont growth, for example through strict controls on their reproduction.

Below we describe these types of symbiont containment and controls, starting with highly compartmentalized examples and moving to less compartmentalized examples.

2.1. Compartmentalization as a containment mechanism

Symbiosomes are the compartments created by scleratinian corals to host *Symbionidium* symbionts (ref. 34, **Table 1**). These compartments create a favourable environment to modulate symbiont physiology and promote photosynthesis [35], while simultaneously allowing the host to control the algal symbiont's growth [36]. Coral host compartments are generally symbiont-specific, and hosts use precise cell-cell recognition to coordinate partner entry [37 but see ref. 38]. Hosts have evolved mechanisms to preferentially expel highly proliferating cells over non-proliferating cells, effectively regulating population densities on a small spatial scale. This means that factors that increase symbiont division rates, such as elevated temperature, simultaneously increase expulsion rates [39].

In insects, containment of microbes in specific structures called bacteriocytes, allow hosts to mediate control over symbiont spread and reproduction (ref. 40, **Table 1**, **Figure 1a**). Many – but not all – insect lineages that rely on microbial symbionts have evolved bacteriocytes, suggesting it is a common evolutionary solution for mediating symbiotic interactions [41]. In theory, bacteriocytes allow hosts to precisely control where and when microbes spread and reproduce, and thus the evolution of these structures can provide a direct benefit to hosts. While bacteriocytes tend to be highly specific, hosting only certain symbiont lineages, the occupancy of these compartments can change over evolutionary time in some insect lineages [42,43]. For example, in Japanese cicadas, recurrent losses of the bacterial symbionts *Hodgkinia* has been mirrored by recruitment of fungal symbiont lineages from the genus *Ophiocordyceps*. In these cases, fungal symbionts are located in the same bacteriome compartments that had hosted *Hodgkinia* - or in some cases - adjacent to it [32].

The physical confinement of microbes to specific host tissue can mediate how partners interact, and even drive the evolution of metabolic dependencies between those partners. As a result, once independent partners may begin to function collectively as a single metabolic unit [40]. In the mouthless catenulid flatworm genus *Paracatenula*, up to 50% of the body volume of some worm species can be composed of symbionts. Over 500 million years of evolution, hosts have evolved to depend on nutritional symbionts to such a degree that they have lost their mouths and digestive tracks. Housed in compartments known as trophosomes – *Paracatenula* symbionts, similar to some insect symbionts, have reduced genomes and are passed directly from parent to off-spring (**Table 1**, ref. 44). In insects, metabolic dependencies tend to be associated with spatially explicit arrangements of the interacting microbes similar to organelles [45,46]. Metabolic co-dependencies are particularly evident in nested symbioses, whereby insects - for example mealy bugs –confine symbionts, which in turn harbour their own symbionts (ref. 47, **Figure 1a**).

If insect bacteriocytes represent the extreme of compartmentalization, the “selective assortment” of symbionts into chemical and physical microenvironments is a less drastic form of confinement practiced by some hosts. It is more fluid, meaning the boundaries of the compartment or not as discrete or permanent as for strict compartmentalisation (**Table 1**), but likewise successful. Historically, the gut (with

the exception of ruminants) was considered an open pipe system, devoid of structured compartments. Recent higher resolution mapping has challenged this view by uncovering spatially explicit sorting of symbiont communities (refs. 48-49, **Figure 1g**). In the vertebrate gut microbiome, for example, bacteria are often contained in compartments characterized by diverse microenvironments differing in their pH, food resources, and O₂ and H₂ gradients [4,50-53]. In the mammal gut, this compartmentalization by microenvironment is reinforced by the immune system. Bacterial recognition via Toll-like receptor signalling from the innate immunity system is key in removing the few bacteria which cross the containment barrier (intestinal lumen into the mucosa) [54]. This function of killing the ‘escaped bacteria’ from the immune system can be compensated by the adaptive immune system if the innate immunity system fails [54]. These mechanisms drive the compartmentalization of bacteria in the gut lumen, which is pivotal for the maintenance of the host-microbiome symbiosis.

Gut compartments in invertebrates likewise play important roles in mediating the benefits of microbial consortia [55,56]. In the honey bee microbiome, microbes degrade distinct molecules in spatially explicit patterns, with major sugar fermenters such as *Gilliamella apicola* in the centre of the lumen, *Lactobacillus spp.* in the distal rectum producing short-chain fatty acids (SCFA), and *Snodgrassella alvi* in the hindgut wall using acetate to maintain a stable O₂ gradient. In other insects, such as termites, gut compartments become even more physically and chemically defined as hosts must employ specific microbes to break down complex materials, such as lignocellulose [57]. In contrast to the strict compartmentalization observed in insect bacteriomes and bacteriocytes, these gut compartments are more fluid in time and space (**Table 1**).

Even in externally open environments, such as skin microbiomes, hosts rely on “compartmentalised control” for skin immunity (**Figure 1e**). Here compartmentalization consists in confining commensal microbes to defined niches, such as hair follicles and sebaceous glands. This spatially explicit confinement allows symbionts to mediate local immunity of the host’s skin [58].

2.2. Compartmentalization facilitates control of partner reproduction

By spatially restricting partners to specific tissues, hosts can control their reproduction. The most effective route is via strict vertical transmission of symbiont lineages in specific compartments [4,8,40,41]. Bacteriocytes are extremely effective in facilitating the transmission of obligate symbionts in ways that resemble the transmission of organelles [59,60]. While in many cases symbiont transmission is directly coupled to the host’s reproductive organs (e.g. refs. 61-62), specialized external compartments and compartmentalising behaviours can also be used to control partner reproduction, especially in cases in which symbionts are hosted extracellularly [63]. This includes attine ant genera that extracellularly harbour their bacterial symbionts in specialized cuticular crypts [64,65], and also symbionts of Pyrrhocoridae bugs, whose reproduction is controlled via egg smearing, symbiont capsules and jelly secretions, respectively (ref. 63 and references therein, **Figure 1f**). In these cases, compartmentalising the symbiont in a specific secretion results in the ability to transmit symbiont lineages with comparable effectiveness as intracellular symbiont transmission [63,66-68]. Recent work has shown that it is not strictly the

mode of transmission that stabilizes cooperation, but rather the outcome of transmission: namely genetic uniformity (high symbiont relatedness) of communities within hosts [16,69].

Compartmentalization can likewise facilitate manipulation of a partner's life-cycle as a means to control reproduction. In the legume-*Rhizobium* nitrogen-fixing symbioses, plant hosts can manipulate individual bacterium housed in autonomous compartments called nodules (**Table 1**) [70]. Some legume hosts are able to induce extreme cell swelling that forces terminal differentiation in their nitrogen fixing rhizobial symbionts. By suppressing *Rhizobia* reproduction in nodule specific compartments, the host is able to actively rewire investment away from symbiont reproduction, and towards into N₂ fixation (**see section 3**). This mechanism has evolved multiple times across the legumes [71,72].

In extreme cases of life-cycle manipulation, the host can use physical confinement to prevent symbionts from entering a reproductive stage at all. This is a necessary adaptation in the symbiosis between Japanese cicadas and *Ophiocordyceps* fungi: reproduction by *Ophiocordyceps* fungi has the potential to directly kill hosts [32]. In other cases, hosts have evolved means to link life-cycle manipulation to environmental conditions. The protist host *Paramecium bursaria*, which compartmentalise individual algal cell within a peri-algal vacuole controls the growth of its *Chorella* algal symbiont according to light levels (ref. 33, **Figure 1b**). The host has evolved means to limit reproduction via acidification of the peri-algal vacuole in low light levels where the partner fails to provide significant benefits [73]. Host regulation of symbiont load may likewise operate via host-triggered symbiont division [74,75] (**see section 3**). Similar mechanisms operate in the Cnidarian-Dinoflagellate symbiosis, whereby hosts can constrain symbiont cell division by acidifying the symbiosome [76].

3. Host control over compartments: discrimination followed by punishments and rewards

Compartmentalization offers the possibility to spatially screen and monitor competing genotypes [77]. In the absence of such fine-tuned discrimination, hosts can only evaluate the collective, rather than individual, performance of their partners. This results in selfish genotypes spreading at the expense of cooperating genotypes [7,15,16]. Below, we discuss how hosts have evolved monitoring platforms to identify differences and discriminate among symbionts (**section 3.1**). We then show how hosts use this information to selectively allocate resources to the best partners, while punishing cheaters (**section 3.2**).

3.1. Compartments allow for discrimination among partners

Discrimination is used in the literature to describe both (i) pre-infection mechanisms of partner choice (i.e. filtering step) and (ii) the post-infection platform of the host to monitor individual partner benefits. Here, we illustrate how compartments are used in the legume-/*Rhizobia* and bobtail squid/*Vibrio* symbioses for pre-infection and post-infection discrimination, and highlight research gaps these fields.

Legume plants can bear tens to hundreds, even thousands, of nodules along their root systems, but each nodule is generally colonized by a single *Rhizobia* (ref. 78, **Figure 1c**). This strict compartmentalization is highly controlled and initiated in legume roots when root hairs curl to encompass a single bacterium to form a nodule [79]. Pre-infection compatibility during these processes is insured by complex signalling and cell membrane receptors [80]. This is a form of partner choice that results in only a specific subset of rhizobia getting into nodules. However, some legume hosts, such as the common bean *Phaseolus vulgaris*, are more promiscuous than others, allowing for several types of rhizobia to form intracellular infections [81,82]. This could increase the chance of infection by less-beneficial rhizobia. An open question is whether the costs of monitoring these more diverse partnerships in nodules are high, and acts as a selection pressure driving legume hosts to evolve specificity in compartmentalization, as is seen more commonly [82]. Hosts allowing promiscuous infections could also face a stronger selection pressure to evolve effective post-infection discrimination (see Section 3.2).

The mechanisms of how hosts monitor individual nodules, once formed, are still largely unknown. New tools, such as reporter plasmids that facilitate high-throughput measurement of N₂ fixation in individual nodules, recently allowed researchers to simultaneously monitor 84 different *Rhizobium leguminosarum* strains in pea hosts [83]. These types of datasets will greatly improve our understanding of the mechanisms of host monitoring because rhizobia fitness can be more precisely linked with N₂ symbiont-provided benefits.

Like in many host-symbiont relationships, symbionts are often under strong selection to subvert host discrimination mechanisms. Rhizobia have evolved a diverse set of tools to prevent hosts from regulating the nodulation process [84]. These include the ability to hyper-proliferate within nodule tissue [85], and to form nodules even when nitrogen from the environment is readily available [86]. In response, hosts can evolve resistance to counter-manipulations, escalating the arms race [85,87,88].

Despite being hugely divergent in their habitat, function, and phylogenetic histories, Sepiolid squid and legume plants have converged on surprisingly similar physical structures for symbiont control. Like legumes, squid house their bioluminescent symbiont bacteria (*Vibrio fischeri*) in specialized nutrient-rich epithelial compartments, known as light organs, where the symbionts reach densities of 10¹¹ cm⁻³ (refs. 89-90, **Figure 1d**). During nocturnal hunting, the squid use light produced by bacteria in these crypts as counter illumination, potentially to hide from predators swimming at a lower depth [91].

Similar to nodules, each crypt is typically, but not always, colonized by a single bacterium, which forms a population of light-producing bacteria within the same day. However, squid hosts get to this single genotype stage in a very different way than legumes. Rather than using strict pre-infection discrimination, Squid hosts use a winnowing process that involves a range of morphological, immunological and biochemical adaptations. This consists of hosts creating a selective environment in which only certain partners succeed [92,93]. Daily ventilation of the entire duct system removes ~90% of all *Vibrio* cells present, and constant environmental sampling by host is thought to act as a selective filter to ensure that compartments are highly controlled [94], and non-luminescent bacteria are eliminated [89,95]. It has

been hypothesized that discrimination against symbiont defectors can be detected upon contact, such that *Vibrio* dark mutants fail to trigger light organ swelling, a key step in the initiation of the symbiosis [96-98] (see **section 3.2**). Similar to rhizobia, *Vibrio* have evolved mechanisms to influence the selection process - for example the ability to form biofilms and hyperaggregate can quantitatively influence how symbionts interact with squid hosts [99].

3.2. Compartmentalization drives rewards and punishments

Hosts monitor symbionts to gather information on the benefits they provide (see **section 3.1**). In theory, this ability to discriminate among simultaneously competing symbionts, allows for a subsequent reaction in which hosts selectively reward and punish partners with greater effectiveness. This can include the preferential parcelling of resources (the “carrot”) or the selective punishment of cheats (the “stick”). In reality, it can be difficult to biologically separate the monitoring/discrimination step of symbiont control from selective rewarding and punishing.

In the legume-*Rhizobia* symbiosis, hosts use discrimination to selectively form the nodule, as well as using both rewards and punishment to mediate rhizobial success inside the nodule. Past work has shown how legumes can couple resource allocation (i.e. sugars) to N₂ fixation rate [70,100,101], and also suffocate rhizobia that fail to fix N₂ [102]. In the sepiolid squid-*Vibrio* symbiosis, hosts rely on high levels of reactive oxygen species (ROS) that can be neutralised by light-producing reactions, allowing squids to link light benefits with symbiont survival and reproduction [90]. In the *Paramecium bursaria* – *Chlorella* symbiosis, the host actively regulates nutrient exchange, such that Ca²⁺ from the host inhibits amino acid uptake into *Chlorella*, whereas host glucose increases the uptake – this is thought to act as selective reward-system for productive symbionts within a single host (refs. 33, 103 and references therein).

The ability to link resource allocation to symbiotic performance is facilitated when a host is able to fully enclose their symbiont partner [104], as found in legume nodules, squid crypts, and peri-algal vacuoles of *Paramecium*. But in some cases, the host only compartmentalises the structure of the symbiont where nutritional exchanges occur (**Table 1, Figure 11**). We call this partial compartmentalisation. In the symbiosis between land plants and arbuscular mycorrhizal (AM) fungi, a plant root will be simultaneously colonized by multiple strains of AM fungi – however the hyphae of each strain will be confined to a membrane-bound host root compartment [105-107]. These compartments are formed when the plasma membrane of the host cell invaginates and proliferates around developing intracellular fungal structures [108]. Arbuscules are the primary sites of nutrient transfer, and their compartmentalization facilitates the discrimination among competing fungal strains [109]. There is evidence that the arbuscules providing less nutrients generally degenerate faster than more profitable arbuscules, leading to the hypothesis that hosts use arbuscules to monitor symbiotic quality and regulate symbiont success [110]. While the system potentially allows hosts to mediate carbon allocation at the level of individual root cells, it does not fully enclose the fungal network. This means the fungus is free to actively move resources to areas of higher plant demand, where it can potentially gain better returns [111], or even find and colonise a less-discriminating host plant [112].

In all these examples, compartmentalization allows hosts to discriminate among partners based on symbiont performance, rather than relying solely on symbiont identity. This may be fundamentally important for the stability of many horizontally transmitted symbioses. Pre-infection mechanisms (i.e. ‘partner choice’) generally rely on signalling cues as indicator of partner quality. Signals, however, are notoriously vulnerable to exploitation or cheating mimicking signals of cooperative competitors [113,114]. A system that uses compartmentalization to monitor and preferentially allocate resources will be more robust to cheating or exploitation [15,88,115]. A critical test of the robustness of post-infection sanctions was demonstrated in a recent experiment whereby pea hosts were colonized by isogenic lines of *Rhizobia* that differed in the expression level of the nitrogen-fixing gene *nifH*. The work showed that pea hosts were unable to identify non-fixing *Rhizobia* at the pre-infection stage, but successfully discriminated against the non-fixing strain after nodule formation by decreasing sugar allocation to nodules formed by this ineffective partner [101].

Scaling up to mutualisms between plants and pollinators, compartmentalization likewise mediate partner rewards. In nursery pollination systems, plants are pollinated exclusively by specific lineages of pollinators in exchange for oviposition within flowers [116-118]. The hatching larvae, which are obligately dependent on the host plant, then consume a subset of the resulting seeds. There is a tension between pollinators and host plants because when a pollinator lays too many eggs on a single flower, a high proportion of seeds will be eaten by pollinator larvae, hampering plant reproduction. In figs, hosts appear to control the number of wasps entering each reproductive compartment (i.e. enclosed fig inflorescence), with numbers of foundress wasps per fruit more clearly reflecting the reproductive interests of the figs compared to the wasps [116]. In Figs, *Yucca* and *Glochidion*, there is some evidence that hosts can selectively abort flowers with high egg loads (**Table 1**; refs. 118-122). This type of compartment-level sanctioning by host plants results in a direct cost to pollinating insects: moths suffer fitness losses as high as 62% in the *Glochidion* mutualism they oviposit into pre-infested flowers [118]. Now future work needs to explore the extent to which this behaviour is based on pre-adaptations versus adaptive responses directly to cheating pollinators. These examples highlight that compartmentalization does not only occur in microbial symbioses (see this section and **Conclusion** for outstanding questions).

3.3. Imperfect discrimination can still be efficient

Above, we have focused on the effectiveness of discrimination at the compartment level. However, discrimination is rarely absolute. It more likely follows a continuum of precision and efficiency. Biologically, this can mean that hosts allocate resources into coarse features (also called “modules”), where symbiotic services are generated rather than directly allocating resources to individual symbionts [123]. While at first sight it appears to be best option, precise sanctioning could not be the rule. This could be because precise sanctioning is costly, or because it is physically impossible, as in the case of the fig-fig-wasp mutualism [124]. Thus, this implies that there could very well be an evolutionarily optimum level well below ‘maximum precision’.

The degree to which symbionts are effectively compartmentalised is likely related to the direct benefits the host receives by controlling (or not controlling) the partner. *Parasponia* plants in the Cannabaceae family are the only known non-legume host to

form N₂ fixing symbioses with rhizobia. The symbiosis has been called “a delicate balance between mutual benefits and parasitic colonization”, largely because of the inability of the host to compartmentalise and control growth of certain inefficient rhizobial strains [125]. In contrast to legume nodules, in which rhizobia are housed in transient organelle-like structures (**Figure 1c**), rhizobia remain in intracellular fixation threads in *Parasponia* nodule cells, and nodules appear more like lateral roots [115]. However imperfect the discrimination, there is evidence that hosts can mediate the success of the intracellular rhizobia when necessary, like under high nitrogen conditions [126].

4. Compartmentalization can prevent within-host conflict

Hosts interact with multitudes of microbes. The coexistence of diverse consortiums is often crucial to nutritional and defensive functions of the host [127-130]. However, mixing of symbiont lineages can create within-host conflict. Within-host conflict means that competitive interactions among the symbionts themselves are detrimental to the host, for example by driving an overall reduction in symbiont populations. This has been shown, for example, in competition among arbuscular mycorrhizal fungi colonizing a single root system: competitive interactions led to both: (i) competitors investing more in accessing host root resources (i.e. which benefits the symbiont) compared to growth strategies dedicated to nutrient foraging (i.e. which benefits the host), and (ii) an overall reduction in mycorrhizal fungal biomass [131]. Theory by Frank [7,8] raised the issue that symbiont mixing can lead to conflict, but also stressed the idea that hosts would not be under strong selection to reduce symbiont diversity, because the benefits of reducing diversity would not be immediate in many systems. However, in cases where hosts realise an immediate benefit to reducing or restricting diversity, these processes can be a strong selection pressure (e.g. ref. 132).

This is further illustrated in examples where hosts use compartments to create selective habitats in which only the effective mutualists thrive. In the bean bug *Riptortus*, highly specialised crypts in the posterior midgut create a selective environment where mutualistic *Burkholderia* bacteria can outcompete non-symbiotic bacteria, effectively functioning as an organ for symbiont sorting [133]. Similarly, African fungus-growing termites actively propagate single variants of their *Termitomyces* symbiont, even though cultures are initially started from genetically variable spores from the habitat. In these cases, the “compartments” are selective habitats created by the host to help mediate competition among symbionts by facilitating the colonization of the preferred symbiont over the less-preferred symbiont (ref. 19, **Table 1**).

In theory, compartmentalization is a powerful way to avoid within-host symbiont conflicts. But as discussed above (**see section 3.3**), imperfect compartmentalization may be common. In legumes, for example, when rhizobial densities in soil are high, nodules can contain more than one founding bacterium, creating ‘mixed nodules’ of multi-genotypes [100,134,135]. New work suggests that mixed nodules are even more common than previously assumed: one study found mixed occupancy in ~20% of nodules, with some nodules containing up to six different strains [83].

The problem of mixed nodules is two-fold. First, competition within a nodule may divert resources away from N₂ fixation and towards competitive antagonism [136],

this potentially includes the production of rhizobial warfare compounds such as bacteriocins [137]. Second, compartmentalization in mixed nodules is less-effective because low-quality strains can benefit from sharing a nodule with a high-quality N₂ fixer, facilitating their spread [138,139]. Again, this is similar to secondary invasion of squid crypts by *Vibrio*, meaning that strict segregation of symbiont lineages is not always possible [140]. In the first case, the reduction of effectiveness in symbiosis is due to within-host conflict, whereas in the second case, the conflict is between the host and the symbiont.

There is evidence, however, in both legume and squid systems that hosts can target mixed compartments, and eliminate competing strains and poor partners. Emerging evidence based on micrographs, suggests that certain legumes (e.g. *Lotus*) can target individual non-N₂ fixing bacteroids in mixed nodules containing both fixing and non-fixing genotypes [139]. There is also evidence that squids can detect and eliminate low-quality strains from mixed crypts [95]. So-called ‘dark mutants’ that fail to produce light, can initially colonize squid crypts, but they decline exponentially reaching undetectable levels within weeks [141]. The mechanistic basis of these process is not yet understood, but the finding underscores the idea that precision in segregation is under strong selection.

5. Compartmentalization is not always the solution

Compartmentalization is a very powerful ‘divide and conquer’ strategy for dealing with multiple partners. However, it is not always the best – or only – evolutionary solution. Hosts could maintain symbiont quality by providing a specific environment to selectively cultivate high quality symbionts [142-146]. In microbiomes, this mechanism works by allowing symbionts to directly compete in ways which winnow out less effective partners [147]. This suggests that within-host conflict (**see section 4**) can be positive for hosts rather than negative in some systems and contexts. While the symbionts are still restricted to specific crypts or structures over which the host has a high level of control, it is the within-host symbiont competition itself that drives selection for the “optimal” symbiont. Are there general patterns for explaining cases where compartmentalization has not evolved in symbiotic partnerships?

First, compartmentalization is only likely to evolve if hosts realise a direct and immediate benefit of compartmentalising their partners [7,8,148]. Second, there are cases where simultaneously hosting multiple partners may be good because partners provide different types of benefits, which are important in different environments or across ontogeny [5, 6]. For instance, in the whistle-thorn acacia (*Vachellia drepanolobium*), four species of symbiotic ants provide distinct mutualistic services across ontogeny, yet they cannot coexist on the same tree [149]. Here, conflict between multiple partner is not suppressed by hosts, and competition among partners even be positive because it allows for selection the most effective symbiont under those certain conditions. Examples include fungus-cultivating ambrosia beetles whereby direct competition among fungal strains helps hosts select for more effective symbionts [143], and toxic warfare among symbionts competing in the guts of bees potentially leading to more beneficial gut communities [150].

Third, there are cases in which the host does not experience costs associated with multiple partners. This is likely because partners are not directly fed by the host, and thus do not represent a cost. In deep sea *Bathymodiolus* mussels, individuals can host as many as 16 co-existing strains of intracellular, sulfur-oxidizing symbionts [21]. The strains, which differ in key functions, feed from the environment (i.e. sulphur seeps) and despite their intracellular nature, are low-cost for mussel hosts. More generally, low-cost partnerships do not face the same selection pressures as those where hosts are providing a large portion of their resources (e.g. 10-20% of fixed carbon to rhizobia) to maintaining symbiotic partners.

6. Conclusion

Compartmentalisation is widespread and fundamental to cooperative partnerships. It allows hosts to: (i) contain symbionts, preventing parasitic invasion and manipulating symbiont reproduction; (ii) discriminate between cooperative and non-cooperative symbionts, enabling rewards and punishment or cessation of the interaction and (iii) reduce within-host conflict. Our framework therefore suggests a number of future research directions. First, we know very little about the selective pressures governing when and what type of compartment evolves – for instance when do we see strict versus fluid compartments evolving (**Table 1**)? Based on our current understanding of the distribution of compartment types, we would expect strict compartmentalisation to evolve where strict control over reproduction and/or controlled resource allocation is selected for? Second, it is unclear whether the precision of compartmentalisation (symbiont genotype versus more modular) depends more on the costs to the host of increased precision, or on the physical constraints of evolving precision in compartmentalized symbioses. Third, our treatment of compartmentalization as a mechanism to reduce within-host conflict has focused on symbiosis with microbes. Whether compartmentalization occurs in mutualisms with macro-organisms and can reduce conflict between different mutualist species is an open question. Answering these questions remains a major task.

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References:

1. Schloissnig S, Arumugam M, Sunagawa S, Mitreva M, Tap J, Zhu A, Waller A, Mende DR, Kultima JR, Martin J, Kota K. 2013 Genomic variation landscape of the human gut microbiome. *Nature* **493**, 45-50. (doi:10.1038/nature11711)
2. Bronstein JL. 2015 ed. *Mutualism*. Oxford University Press, USA.
3. Martin FM, Uroz S, Barker DG. 2017 Ancestral alliances: plant mutualistic symbioses with fungi and bacteria. *Science* **356**, eaad4501. (Doi: 10.1126/science.aad4501).
4. Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. 2017 The evolution of the host microbiome as an ecosystem on a leash. *Nature* **548**, 43-51. (doi:10.1038/nature23292)

5. Batstone RT, Carscadden KA, Afkhami ME, Frederickson ME. 2018 Using niche breadth theory to explain generalization in mutualisms. *Ecology* **99**,1039-1050.
6. Chomicki G, Weber M, Antonelli A, Bascompte J, Kiers ET. 2019 The impact of mutualisms on species richness. *Trends Ecol. Evol.* **34**, 698-711. (doi.org/10.1016/j.tree.2019.03.003)
7. Frank SA. 1994 Genetics of mutualism: the evolution of altruism between species. *J. Theor. Biol.* **170**, 393-400. (doi.org/10.1006/jtbi.1994.1200)
8. Frank, S.A. Host–symbiont conflict over the mixing of symbiotic lineages. *Proc. Royal Soc. B* **263**, 339-344 (1996). (doi.org/10.1098/rspb.1996.0052)
9. Frank SA. 1996 Host control of symbiont transmission: the separation of symbionts into germ and soma. *Am. Nat.* **148**, 1113-1124. (doi.org/10.1086/285974)
10. Frank SA. 1997. Models of symbiosis. *Am. Nat.* **150**, S80-s99. (doi.org/10.1086/286051)
11. Frank SA. 2003 Repression of competition and the evolution of cooperation. *Evolution* **57**, 693-705. (doi.org/10.1111/j.0014-3820.2003.tb00283.x)
12. Foster KR, Wenseleers, T. A general model for the evolution of mutualisms. *J. Evol. Biol.* **19**, 1283-1293 (2006). (doi.org/10.1111/j.1420-9101.2005.01073.x)
13. Ghoul, M., Griffin, A.S. and West, S.A., 2014. Toward an evolutionary definition of cheating. *Evolution*, 68(2), pp.318-331.
14. Chomicki G, Kiers ET, Renner SS. The evolution of mutualistic dependence. *Annual Review of Ecology, Evolution, and Systematics* 51 (in press).
15. West SA, Kiers ET, Simms EL, Denison RF. 2002 Sanctions and mutualism stability: why do rhizobia fix nitrogen?. *Proc. Royal Soc. B* **269**, 685-694. (doi.org/10.1098/rspb.2001.1878)
16. Leeks A, dos Santos M, West SA. 2019 Transmission, relatedness, and the evolution of cooperative symbionts. *J. Evol. Biol.* **32**, 1036-1045. (doi.org/10.1111/jeb.13505)
17. Fisher RM, Henry LM, Cornwallis CK, Kiers ET, West SA. 2017 The evolution of host-symbiont dependence. *Nat. Comm.* **8**, 15973. (doi:10.1038/ncomms15973)
18. Sachs JL, Wilcox TP. 2005 A shift to parasitism in the jellyfish symbiont *Symbiodinium microadriaticum*. *Proc. Royal Soc. B* **273**, 425-429. (doi: 10.1098/rspb.2005.3346)
19. Aanen DK, Henrik H, Debets AJ, Kerstes NA, Hoekstra RF, Boomsma JJ. 2009 High symbiont relatedness stabilizes mutualistic cooperation in fungus-growing termites. *Science* **326**, 1103-1106. (Doi: 10.1126/science.1173462)
20. Engel P, Stepanauskas R, Moran NA. 2014 Hidden diversity in honey bee gut symbionts detected by single-cell genomics. *PLoS Genet.* **10**, e1004596. (doi.org/10.1371/journal.pgen.1004596)
21. Ansoerge R, Romano S, Sayavedra L, Porras MÁG, Kupczok A, Tegetmeyer HE, Dubilier N, Petersen J. 2019 Functional diversity enables multiple symbiont strains to coexist in deep-sea mussels. *Nat. Microbiol.* **4**, 2487-2497. (doi:10.1038/s41564-019-0572-9)

22. Maynard Smith J, Szathmáry E, *The Major Transitions in Evolution*. Oxford Univ. Press, Oxford, 1995.
23. Yamamura N. 1993. Vertical transmission and evolution of mutualism from parasitism. *Theor. Pop. Biol.* **44**, 95-109. (doi.org/10.1006/tpbi.1993.1020)
24. Salem H, Onchuru TO, Bauer E, Kaltenpoth M. 2015. Symbiont transmission entails the risk of parasite infection. *Biol. Lett.* **11**, 20150840. (doi.org/10.1098/rsbl.2015.0840)
25. King KC, Brockhurst MA, Vasieva O, Paterson S, Betts A, Ford SA, Frost CL, Horsburgh MJ, Haldenby S, Hurst GD. 2016 Rapid evolution of microbe-mediated protection against pathogens in a worm host. *ISME J.* **10**, 1915-1924. (doi.org/10.1038/ismej.2015.259)
26. Shapiro JW, Turner PE. 2018 Evolution of mutualism from parasitism in experimental virus populations. *Evolution* **72**, 707-712. (doi.org/10.1111/evo.13440)
27. Tso GHW, Reales-Calderon JA, Tan ASM, Sem X, Le GTT, Tan TG, Lai GC, Srinivasan KG, Yurieva M, Liao W, Poidinger M. 2018 Experimental evolution of a fungal pathogen into a gut symbiont. *Science* **362**, 589-595. (DOI: 10.1126/science.aat0537)
28. Law R, Dieckmann U. 1998 Symbiosis through exploitation and the merger of lineages in evolution. *Proc. R. Soc. B* **265**, 1245-1253. (doi.org/10.1098/rspb.1998.0426)
29. Spatafora JW, Sung GH, Sung JM, Hywel-Jones NL, White Jr JF. 2007 Phylogenetic evidence for an animal pathogen origin of ergot and the grass endophytes. *Mol. Ecol.* **16**, 1701-1711. (doi.org/10.1111/j.1365-294X.2007.03225.x)
30. Sachs JL, Skophammer RG, Regus JU. 2011 Evolutionary transitions in bacterial symbiosis. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 10800-10807. (https://doi.org/10.1073/pnas.1100304108)
31. Zug R, Hammerstein P. 2015 Bad guys turned nice? A critical assessment of Wolbachia mutualisms in arthropod hosts. *Biol. Rev.* **90**, 89-111. (doi.org/10.1111/brv.12098)
32. Matsuura Y, Moriyama M, Łukasik P, Vanderpool D, Tanahashi M, Meng X Y, McCutcheon JP, Fukatsu T. 2018 Recurrent symbiont recruitment from fungal parasites in cicadas. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E5970-E5979. (doi.org/10.1073/pnas.1803245115)
33. Sørensen ME, Lowe CD, Minter EJ, Wood AJ, Cameron D, Brockhurst MA. 2019 The role of exploitation in the establishment of mutualistic microbial symbioses. *FEMS Microbiol. Lett.* **366**, fnz148. (doi.org/10.1093/femsle/fnz148)
34. Fransolet D, Roberty S, Plumier JC. 2012. Establishment of endosymbiosis: the case of cnidarians and *Symbiodinium*. *J. Exp. Mar. Biol. Ecol.* **420**, 1-7. (doi.org/10.1016/j.jembe.2012.03.015)
35. Barott KL, Venn AA, Perez SO, Tambutté S, Tresguerres M. 2015. Coral host cells acidify symbiotic algal microenvironment to promote photosynthesis. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 607-612. (doi.org/10.1073/pnas.1413483112)
36. Wilkerson FP, Kobayashi D, Muscatine L. 1988 Mitotic index and size of symbiotic algae in Caribbean reef corals. *Coral Reefs* **7**, 29-36. (doi.org/10.1007/BF00301979)

37. Wood-Charlson EM, Hollingsworth LL, Krupp DA, Weis VM. 2006 Lectin/glycan interactions play a role in recognition in a coral/dinoflagellate symbiosis. *Cellular Microbiol.* **8**, 1985-1993. (doi.org/10.1111/j.1462-5822.2006.00765.x)
38. Silverstein RN, Correa AM, Baker AC. 2012 Specificity is rarely absolute in coral–algal symbiosis: implications for coral response to climate change. *Proc. Roy. Soc. B* **279**, 2609-2618. (doi.org/10.1098/rspb.2012.0055)
39. Baghdasarian G, Muscatine L. 2000. Preferential expulsion of dividing algal cells as a mechanism for regulating algal-cnidarian symbiosis. *Biol. Bull.* **199**, 278-286. (doi.org/10.2307/1543184)
40. Douglas AE. 2016. How multi-partner endosymbioses function. *Nat. Rev. Microbiol.* **14**, 731. (doi:10.1038/nrmicro.2016.151)
41. Douglas, A.E., 2010. *The symbiotic habit*. Princeton University Press.
42. Koga R, Bennett GM, Cryan JR, Moran NA. 2013. Evolutionary replacement of obligate symbionts in an ancient and diverse insect lineage. *Environ. Microbiol.* **15**, 2073-2081. (http://doi.org/10.1111/1462-2920.12121)
43. Koga R, Moran NA. 2014. Swapping symbionts in spittlebugs: evolutionary replacement of a reduced genome symbiont. *ISME J.* **8**, 1237. (doi:10.1038/ismej.2013.235)
44. Gruber-Vodicka HR, Dirks U, Leisch N, Baranyi C, Stoecker K, Bulgheresi S, Heindl NR, Horn M, Lott C, Loy A, Wagner M. 2011. *Paracatenula*, an ancient symbiosis between thiotrophic Alphaproteobacteria and catenulid flatworms. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 12078-12083. (https://doi.org/10.1073/pnas.1105347108)
45. McCutcheon JP, Von Dohlen CD. 2011. An interdependent metabolic patchwork in the nested symbiosis of mealybugs. *Curr. Biol.* **21**, 1366-1372. (http://doi.org/10.1016/j.cub.2011.06.051)
46. López-Madrigal S, Latorre A, Porcar M, Moya A, Gil R. 2013. Mealybugs nested endosymbiosis: going into the ‘matryoshka’ system in *Planococcus citri* in depth. *BMC Microbiol.* **13**, 74. (<http://doi.org/10.1186/1471-2180-13-74>)
47. Husnik F, McCutcheon JP. 2016. Repeated replacement of an intrabacterial symbiont in the tripartite nested mealybug symbiosis. *Proc. Natl. Acad. Sci. U.S.A.* **113**, E5416-E5424. (<http://doi.org/10.1073/pnas.1603910113>)
48. Weimer PJ. 2015 Redundancy, resilience, and host specificity of the ruminal microbiota: implications for engineering improved ruminal fermentations. *Front. Microbiol.* **6**, 296. (http://doi.org/10.3389/fmicb.2015.00296)
49. Welch JLM, Rossetti BJ, Rieken CW, Dewhirst FE, Borisy GG. 2016 Biogeography of a human oral microbiome at the micron scale. *Proc. Natl. Acad. Sci. U.S.A.* **113**, E791-E800. (<http://doi.org/10.1073/pnas.1522149113>)
50. Caldara M, Friedlander RS, Kavanaugh NL, Aizenberg J, Foster KR, Ribbeck K. 2012. Mucin biopolymers prevent bacterial aggregation by retaining cells in the free-swimming state. *Curr. Biol.* **22**, 2325-2330. (http://doi.org/10.1016/j.cub.2012.10.028)
51. Hooper LV, Littman DR, Macpherson AJ. 2012 Interactions between the microbiota and the immune system. *Science* **336**, 1268-1273. (Doi: 10.1126/science.1223490)

52. Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH. 2013 Going back to the roots: the microbial ecology of the rhizosphere. *Nat. Rev. Microbiol.* **11**, 789–799. (<http://doi.org/10.1038/nrmicro3109>)
53. Hacquard S, Garrido-Oter R, González A, Spaepen S, Ackermann G, Lebeis S, McHardy AC, Dangl JL, Knight R, Ley R, Schulze-Lefert P. 2015 Microbiota and host nutrition across plant and animal kingdoms. *Cell Host Microbe* **17**, 603–616. (<http://doi.org/10.1016/j.chom.2015.04.009>)
54. Slack E, Hapfelmeier S, Stecher B, Velykoredko Y, Stoel M, Lawson MA, Geuking MB, Beutler B, Tedder TF, Hardt WD, Bercik P. 2009 Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. *Science* **325**, 617–620. (Doi: 10.1126/science.1172747)
55. Zheng H, Powell JE, Steele MI, Dietrich C, Moran NA. 2017 Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 4775–4780. (<http://doi.org/10.1073/pnas.1701819114>)
56. Zheng H, Perreau J, Powell JE, Han B, Zhang Z, Kwong WK, Tringe SG, Moran NA. 2019. Division of labor in honey bee gut microbiota for plant polysaccharide digestion. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 25909–25916. (<http://doi.org/10.1073/pnas.1916224116>)
57. Mikaelian A, Meuser K, Brune A. 2017 Microenvironmental heterogeneity of gut compartments drives bacterial community structure in wood-and humus-feeding higher termites. *FEMS Microbiol. Ecol.* **93**, fiw210. (<https://doi.org/10.1093/femsec/fiw210>)
58. Naik S, Bouladoux N, Wilhelm C, Molloy MJ, Salcedo R, Kastenmuller W, Deming C, Quinones M, Koo L, Conlan S, Spencer S. 2012 Compartmentalized control of skin immunity by resident commensals. *Science* **337**, 1115–1119. (Doi: 10.1126/science.1225152)
59. Koga R, Meng XY, Tsuchida T, Fukatsu T. 2012 Cellular mechanism for selective vertical transmission of an obligate insect symbiont at the bacteriocyte–embryo interface. *Proc. Natl. Acad. Sci. U.S.A.* **109**, E1230–E1237. (<http://doi.org/10.1073/pnas.1119212109>)
60. Keeling PJ, McCutcheon JP. 2017 Endosymbiosis: the feeling is not mutual. *J. Theor. Biol.* **434**, 75–79. (<http://doi.org/10.1016/j.jtbi.2017.06.008>)
61. Feldhaar H, Straka J, Krischke M, Berthold K, Stoll S, Mueller MJ, Gross R. 2007. Nutritional upgrading for omnivorous carpenter ants by the endosymbiont *Blochmannia*. *BMC Biol.* **5**, 48. (doi:10.1186/1741-7007-5-48)
62. Perlmutter JJ, Bordenstein SR. 2020 Microorganisms in the reproductive tissues of arthropods. *Nat. Rev. Gen.* (<https://doi.org/10.1038/s41579-019-0309-z>).
63. Salem H, Florez L, Gerardo N, Kaltenpoth M. 2015 An out-of-body experience: the extracellular dimension for the transmission of mutualistic bacteria in insects. *Proc. Roy. Soc. B* **282**, 20142957. (<http://doi.org/10.1098/rspb.2014.2957>)
64. Currie CR, Poulsen M, Mendenhall J, Boomsma JJ, Billen J. 2006 Coevolved crypts and exocrine glands support mutualistic bacteria in fungus-growing ants. *Science* **311**, 81–83. (doi:10.1126/science.1119744).
65. Li H, Sosa-Calvo J, Horn HA, Pupo MT, Clardy J, Rabeling C, Schultz TR, Currie CR. 2018 Convergent evolution of complex structures for ant–bacterial defensive symbiosis in fungus-farming ants. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 10720–10725. (doi.org/10.1073/pnas.1809332115)

66. Hosokawa T, Kikuchi Y, Nikoh N, Shimada M, Fukatsu T. 2006 Strict host-symbiont cospeciation and reductive genome evolution in insect gut bacteria. *PLoS Biol.* **4**, e337. (<https://doi.org/10.1371/journal.pbio.0040337>)
67. Kikuchi Y, Hosokawa T, Nikoh N, Meng XY, Kamagata Y, Fukatsu T. 2009 Host-symbiont co-speciation and reductive genome evolution in gut symbiotic bacteria of acanthosomatid stinkbugs. *BMC Biol.* **7**, 2. (doi:10.1186/1741-7007-7-2)
68. Kaiwa N, Hosokawa T, Nikoh N, Tanahashi M, Moriyama M, Meng XY, Maeda T, Yamaguchi K, Shigenobu S, Ito M, Fukatsu T. 2014 Symbiont-supplemented maternal investment underpinning host's ecological adaptation. *Curr. Biol.* **24**, 2465-2470. (<https://doi.org/10.1016/j.cub.2014.08.065>)
69. Kenkel CD, Bay LK. 2018 Exploring mechanisms that affect coral cooperation: symbiont transmission mode, cell density and community composition. *PeerJ* **6**, e6047. (<https://doi.org/10.7717/peerj.6047>)
70. Sachs JL, Quides KW, Wendlandt CE. 2018. Legumes versus rhizobia: a model for ongoing conflict in symbiosis. *New. Phytol.* **219**, 1199-1206. (<https://doi.org/10.1111/nph.15222>)
71. Mergaert P, Uchiumi T, Alunni B, Evanno G, Cheron A, Catrice O, Mausset AE, Barloy-Hubler F, Galibert F, Kondorosi A, Kondorosi E. 2006. Eukaryotic control on bacterial cell cycle and differentiation in the Rhizobium-legume symbiosis. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 5230-5235. (<https://doi.org/10.1073/pnas.0600912103>)
72. Oono R, Denison RF. 2010. Comparing symbiotic efficiency between swollen versus nonswollen rhizobial bacteroids. *Plant Phys.* **154**, 1541-1548. (<https://doi.org/10.1104/pp.110.163436>)
73. Lowe CD, Minter EJ, Cameron DD, Brockhurst MA. 2016 Shining a light on exploitative host control in a photosynthetic endosymbiosis. *Curr. Biol.* **26**, 207-211. (<https://doi.org/10.1016/j.cub.2015.11.052>)
74. Takahashi T, Shirai Y, Kosaka T, Hosoya H. 2007 Arrest of cytoplasmic streaming induces algal proliferation in green paramecia. *PLoS One* **2**, e1352. (<https://doi.org/10.1371/journal.pone.0001352>)
75. Minter EJ, Lowe CD, Sørensen ME, Wood AJ, Cameron DD, Brockhurst MA. 2018 Variation and asymmetry in host-symbiont dependence in a microbial symbiosis. *BMC Evol. Biol.* **18**, 108. (<http://doi.org/10.1186/s12862-018-1227-9>)
76. Davy SK, Allemand D, Weis VM. 2012 Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiol. Mol. Biol. Rev.* **76**, 229-261. (DOI: 10.1128/MMBR.05014-11)
77. Kiers ET, Denison RF. 2008 Sanctions, cooperation, and the stability of plant-rhizosphere mutualisms. *Ann. Rev. Ecol. Evol. Syst.* **39**, 215-236. (<https://doi.org/10.1073/pnas.1809332115>)
78. Denison RF, Kiers ET. 2011. Life histories of symbiotic rhizobia and mycorrhizal fungi. *Curr. Biol.* **21**, R775-R785. (<https://doi.org/10.1016/j.cub.2011.06.018>)
79. Parniske M. 2018 Uptake of bacteria into living plant cells, the unifying and distinct feature of the nitrogen-fixing root nodule symbiosis. *Curr. Op. Plant Biol.* **44**, 164-174. (<https://doi.org/10.1016/j.pbi.2018.05.016>)

80. Kawaharada Y *et al.* 2015. Receptor-mediated exopolysaccharide perception controls bacterial infection. *Nature* **523**, 308–312.
(<https://doi.org/10.1038/nature14611>)
81. Arthikala MK, Sánchez-López R, Nava N, Santana O, Cárdenas L, Quinto C. 2014. *RbohB*, a *Phaseolus vulgaris* NADPH oxidase gene, enhances symbiosome number, bacteroid size, and nitrogen fixation in nodules and impairs mycorrhizal colonization. *New Phytol.* **202**, 886-900.
(<https://doi.org/10.1111/nph.12714>)
82. Wang Q, Liu J, Zhu H. 2018. Genetic and molecular mechanisms underlying symbiotic specificity in legume-rhizobium interactions. *Front. Plant Sci.* **9**, 313. (<https://doi.org/10.3389/fpls.2018.00313>)
83. Mendoza-Suárez MA, Geddes BA, Sánchez-Cañizares C, Ramírez-González RH, Kirchhelle C, Jorin B, Poole PS. 2020. Optimizing Rhizobium-legume symbioses by simultaneous measurement of rhizobial competitiveness and N₂ fixation in nodules. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 9822-9831.
(<https://doi.org/10.1073/pnas.1921225117>)
84. Ma WB, Penrose DM, Glick BR. 2002 Strategies used by rhizobia to lower plant ethylene levels and increase nodulation. *Can. J. Microbiol.* **48**, 947–954. (DOI: 10.1139/w02-100)
85. Price PA, Tanner HR, Dillon BA, Shabab M, Walker GC, Griffiths JS. 2015 Rhizobial peptidase hrrP cleaves host-encoded signaling peptides and mediates symbiotic compatibility. *Proc. Natl Acad. Sci. USA* **112**, 15244–15249. (doi: 10.1073/pnas.1417797112)
86. Nguyen HP, Miwa H, Obirih-Opareh J, Suzaki T, Yasuda M, Okazaki S. 2019 Novel rhizobia exhibit superior nodulation and biological nitrogen fixation even under high nitrate concentrations. *FEMS Microbiol. Ecol.* **96**, fiz184.
(<https://doi.org/10.1093/femsec/fiz184>)
87. Yasuda M, Miwa H, Masuda S, Takebayashi Y, Sakakibara H, Okazaki S. 2016 Effector-triggered immunity determines host genotype-specific incompatibility in legume-rhizobium symbiosis. *Plant Cell Physiol.* **57**, 1791–1800. (DOI: 10.1093/pcp/pcw104)
88. Gano-Cohen KA, Wendlandt CE, Stokes PJ, Blanton MA, Quides KW, Zomorrodian A, Adinata ES, Sachs JL. 2019 Interspecific conflict and the evolution of ineffective rhizobia. *Ecol. Lett.* **22**, 914-924.
(<https://doi.org/10.1111/ele.13247>)
89. Visick KL, Foster J, Doino J, McFall-Ngai M, Ruby EG. 2000 *Vibrio fischeri* lux genes play an important role in colonization and development of the host light organ. *J. Bacteriol.* **182**, 4578-4586. (DOI: 10.1128/jb.182.16.4578-4586.2000)
90. Nyholm SV, McFall-Ngai M. 2004 The winnowing: establishing the squid–*Vibrio* symbiosis. *Nat. Rev. Microbiol.* **2**, 632-642. (doi:10.1038/nrmicro957)
91. Nyholm SV, Nishiguchi MK. 2008 The evolutionary ecology of a sepiolid squid-*Vibrio* association: from cell to environment. *Vie et milieu* (Paris, France: 1980) **58**, 175.
92. McFall-Ngai M. 2014 Divining the essence of symbiosis: insights from the squid-vibrio model. *PLoS Biol.* **12**, e1001783.
(<http://doi.org/10.1371/journal.pbio.1001783>)
93. Kremer N, Schwartzman J, Augustin R, Zhou L, Ruby EG, Hourdez S, McFall-Ngai MJ. 2014 The dual nature of haemocyanin in the establishment

- and persistence of the squid–vibrio symbiosis. *Proc. Roy. Soc. B* **281**, 20140504. (<http://doi.org/10.1098/rspb.2014.0504>)
94. Schwartzman JA, Ruby EG. 2016 Stress as a normal cue in the symbiotic environment. *Trends Microbiol.* **24**, 414-424. (<http://doi.org/10.1016/j.tim.2016.02.012>)
 95. Tong D, Rozas NS, Oakley TH, Mitchell J, Colley NJ, McFall-Ngai MJ. 2009 Evidence for light perception in a bioluminescent organ. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 9836-9841. (<https://doi.org/10.1073/pnas.0904571106>)
 96. Montgomery MK, McFall-Ngai M. 1994 Bacterial symbionts induce host organ morphogenesis during early postembryonic development of the squid *Euprymna scolopes*. *Development* **120**, 1719-1729.
 97. Ruby EG, McFall-Ngai MJ. 1999 Oxygen-utilizing reactions and symbiotic colonization of the squid light organ by *Vibrio fischeri*. *Trends Microbiol.* **7**, 414-420. ([http://doi.org/10.1016/S0966-842X\(99\)01588-7](http://doi.org/10.1016/S0966-842X(99)01588-7))
 98. Miyashiro T, Ruby EG. 2012 Shedding light on bioluminescence regulation in *Vibrio fischeri*. *Mol. Microbiol.* **84**, 795-806. (<http://doi.org/10.1111/j.1365-2958.2012.08065.x>)
 99. Koehler, S., Gaedeke, R., Thompson, C., Bongrand, C., Visick, K.L., Ruby, E. and McFall-Ngai, M., 2019. The model squid–vibrio symbiosis provides a window into the impact of strain-and species-level differences during the initial stages of symbiont engagement. *Environmental microbiology*, 21(9), pp.3269-3283. (<https://doi.org/10.1111/1462-2920.14392>)
 100. Sachs JL, Russell JE, Lii YE, Black KC, Lopez G, Patil AS. 2010 Host control over infection and proliferation of a cheater symbiont. *J. Evol. Biol.* **23**, 1919-1927. (<https://doi.org/10.1111/j.1420-9101.2010.02056.x>)
 101. Westhoek A, Field E, Rehling F, Mulley G, Webb I, Poole PS, Turnbull LA. 2017 Policing the legume-Rhizobium symbiosis: a critical test of partner choice. *Sci. Rep.* **7**, 1419. (<http://doi.org/10.1038/s41598-017-01634-2>)
 102. Kiers ET, Rousseau RA, West SA, Denison RF. 2003 Host sanctions and the legume–rhizobium mutualism. *Nature* **425**, 78-81. (doi:10.1038/nature01931)
 103. Sørensen ME, Wood AJ, Minter EJ, Lowe CD, Cameron DD, Brockhurst MA. 2020 Comparison of independent evolutionary origins reveals both convergence and divergence in the metabolic mechanisms of symbiosis. *Curr. Biol.* (<https://doi.org/10.1016/j.cub.2019.11.053>)
 104. Kiers ET, West SA. 2016 Evolution: welcome to symbiont prison. *Curr. Biol.* **26**, R66-R68. (<https://doi.org/10.1016/j.cub.2015.12.009>)
 105. Öpik M, Davison J, Moora M, Zobel M. 2013 DNA-based detection and identification of Glomeromycota: the virtual taxonomy of environmental sequences. *Botany* **92**, 135-147. (<https://doi.org/10.1139/cjb-2013-0110>)
 106. Werner GD, Kiers ET. 2015 Partner selection in the mycorrhizal mutualism. *New. Phytol.* **205**, 1437-1442. (<https://doi.org/10.1111/nph.13113>)
 107. Harrison MJ, Ivanov S. 2017. Exocytosis for endosymbiosis: membrane trafficking pathways for development of symbiotic membrane compartments. *Curr. Op. Plant Biol.* **38**, 101-108. (<https://doi.org/10.1016/j.pbi.2017.04.019>)
 108. Balestrini R, Bonfante P. 2014 Cell wall remodeling in mycorrhizal symbiosis: a way towards biotrophism. *Front. Plant Sci.* **5**, 237. (<https://doi.org/10.3389/fpls.2014.00237>)

109. Noë R, Kiers ET. 2018 Mycorrhizal markets, firms, and co-ops. *Trends. Ecol. Evol.* (<https://doi.org/10.1016/j.tree.2018.07.007>)
110. Limpens E, Geurts R. 2014 Plant-driven genome selection of arbuscular mycorrhizal fungi. *Mol. Plant Pathol.* **15**, 531– 534. (doi: 10.1111/mpp.12149)
111. Whiteside MD, Werner GD, Caldas VE, van't Padjé A, Dupin SE, Elbers B, Bakker M, Wyatt GA, Klein M, Hink MA, Postma M. 2019 Mycorrhizal fungi respond to resource inequality by moving phosphorus from rich to poor patches across networks. *Curr. Biol.* **29**, 2043-2050. (<https://doi.org/10.1016/j.cub.2019.04.061>)
112. Grman E. 2012 Plant species differ in their ability to reduce allocation to non-beneficial arbuscular mycorrhizal fungi. *Ecology* **93**, 711-718. (<https://doi.org/10.1890/11-1358.1>)
113. Edwards DP, Yu DW. 2007 The roles of sensory traps in the origin, maintenance, and breakdown of mutualism. *Behav. Ecol. Sociobiol.* **61**, 1321– 1327. (<https://doi.org/10.1007/s00265-007-0369-3>)
114. van't Padjé A, Whiteside MD, Kiers ET. 2016 Signals and cues in the evolution of plant–microbe communication. *Curr. Op. Plant Biol.* **32**, 47-52. (<https://doi.org/10.1016/j.pbi.2016.06.006>)
115. Behm JE, Geurts R, Kiers ET. 2014 *Parasponia*: a novel system for studying mutualism stability. *Trends Plant Sci.* **19**, 757-763. (<https://doi.org/10.1016/j.tplants.2014.08.007>)
116. Herre EA. 1989 Coevolution of reproductive characteristics in 12 species of New World figs and their pollinator wasps. *Experientia* **45**, 637-647. (<https://doi.org/10.1007/BF01975680>)
117. Pellmyr O. 2003 Yuccas, yucca moths, and coevolution: a review. *Ann. Miss. Bot. Gard.* **90**, 35-55. (DOI: 10.2307/3298524)
118. Goto R, Okamoto T, Kiers ET, Kawakita A, Kato M. 2010 Selective flower abortion maintains moth cooperation in a newly discovered pollination mutualism. *Ecol. Lett.* **13**, 321-329. (<https://doi.org/10.1111/j.1461-0248.2009.01425.x>)
119. Pellmyr O, Huth CJ. 1994 Evolutionary stability of mutualism between yuccas and yucca moths. *Nature* **372**, 257-260. (doi:10.1038/372257a0)
120. Addicott, J. F., & Bao, T. (1999). Limiting the costs of mutualism: multiple modes of interaction between yuccas and yucca moths. *Proc. R. Soc. B* **266**, 197-202. (<https://doi.org/10.1098/rspb.1999.0622>)
121. Shapiro J, Addicott JF. 2004 Re-evaluating the role of selective abscission in moth/yucca mutualisms. *Oikos* **105**, 449-460. (<https://doi.org/10.1111/j.0030-1299.2004.12681.x>)
122. Jandér KC, Herre EA. 2010 Host sanctions and pollinator cheating in the fig tree–fig wasp mutualism. *Proc. Roy. Soc. B* **277**, 1481-1488. (<https://doi.org/10.1098/rspb.2009.2157>)
123. Steidinger BS, Bever JD. 2016. Host discrimination in modular mutualisms: a theoretical framework for meta-populations of mutualists and exploiters. *Proc. R. Soc. B* **283**: 20152428. (<https://doi.org/10.1098/rspb.2015.2428>)
124. Jandér CK, Herre EA, Simms EL. 2012. Precision of host sanctions in the fig tree–fig wasp mutualism: consequences for uncooperative symbionts. *Ecol. Lett.* **15**, 1362-1369. (<https://doi.org/10.1111/j.1461-0248.2012.01857.x>)

125. Op den Camp RH, Polone E, Fedorova E, Roelofsen W, Squartini A, Op den Camp HJ, Bisseling T, Geurts R. 2012. Nonlegume *Parasponia andersonii* deploys a broad rhizobium host range strategy resulting in largely variable symbiotic effectiveness. *Mol. Plant Microbe Interact.* **25**, 954-963. (<https://doi.org/10.1094/MPMI-11-11-0304>)
126. Dupin, S.E., Geurts, R. and Kiers, E.T., 2020. The non-legume *Parasponia andersonii* mediates the fitness of nitrogen-fixing rhizobial symbionts under high nitrogen conditions. *Frontiers Plant Sci.* **10**, 1779. (<https://doi.org/10.3389/fpls.2019.01779>)
127. Ursell LK, Treuren WV, Metcalf JL, Pirrung M, Gewirtz A, Knight R. 2013 Replenishing our defensive microbes. *Bioessays* **35**, 810-817. (<https://doi.org/10.1002/bies.201300018>)
128. Smith AH, Łukasik P, O'Connor MP, Lee A, Mayo G, Drott MT, Doll, S, Tuttle R, Disciullo RA, Messina A, Oliver KM. 2015 Patterns, causes and consequences of defensive microbiome dynamics across multiple scales. *Mol. Ecol.* **24**, 1135-1149. (<https://doi.org/10.1111/mec.13095>)
129. Pallister T, Jackson MA, Martin TC, Zierer J, Jennings A, Mohny RP, MacGregor A, Steves CJ, Cassidy A, Spector TD, Menni C. 2017 Hippurate as a metabolomic marker of gut microbiome diversity: Modulation by diet and relationship to metabolic syndrome. *Sci. Rep.* **7**, 13670. (<https://doi.org/10.1038/s41598-017-13722-4>)
130. Valdes AM, Walter J, Segal E, Spector TD. 2018 Role of the gut microbiota in nutrition and health. *BMJ* **361**, k2179. (doi: <https://doi.org/10.1136/bmj.k2179>)
131. Engelman DJ, Behm JE, Kiers ET. 2014 Intense competition between arbuscular mycorrhizal mutualists in an in vitro root microbiome negatively affects total fungal abundance. *Mol. Ecol.* **23**, 1584–1593. (<https://doi.org/10.1111/mec.12451>)
132. Poulsen M, Boomsma JJ. 2005 Mutualistic fungi control crop diversity in fungus-growing ants. *Science* **307**, 741-744. (DOI: 10.1126/science.1106688)
133. Itoh H, Jang S, Takeshita K, Ohbayashi T, Ohnishi N, Meng XY, Mitani Y, Kikuchi Y. 2019 Host–symbiont specificity determined by microbe–microbe competition in an insect gut. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 22673-22682. (<https://doi.org/10.1073/pnas.1912397116>)
134. Denison RF. 2000 Legume sanctions and the evolution of symbiotic cooperation by rhizobia. *Am. Nat.* **156**, 567-576. (<https://doi.org/10.1086/316994>)
135. Gage DJ. 2002 Analysis of infection thread development using Gfp- and DsRed-expressing *Sinorhizobium meliloti*. *J. Bacteriol.* **184**, 7042-7046. (DOI: 10.1128/JB.184.24.7042-7046.2002)
136. Wielbo, J., Kuske, J., Marek-Kozaczuk, M. and Skorupska, A. 2010. The competition between *Rhizobium leguminosarum* bv. *viciae* strains progresses until late stages of symbiosis. *Plant Soil* **337**, 125-135. (<https://doi.org/10.1007/s11104-010-0510-3>)
137. Oresnik IJ, Twelker S, Hynes MF. 1999. Cloning and characterization of a *Rhizobium leguminosarum* gene encoding a bacteriocin with similarities to RTX toxins. *Appl Environ Microbiol* **65**, 2833–2840. (Doi: 10.1128/AEM.65.7.2833-2840.1999)

138. Kier ET, Ratcliff WC, Denison RF. 2013 Single-strain inoculation may create spurious correlations between legume fitness and rhizobial fitness. *New Phytol.* **198**, 4-6. (DOI : 10.1111/nph.12015)
139. Regus JU, Quides KW, O'Neill MR, Suzuki R, Savory EA, Chang JH, Sachs JL. 2017 Cell autonomous sanctions in legumes target ineffective rhizobia in nodules with mixed infections. *Am. J. Bot.* **104**, 1299-1312. (<https://doi.org/10.3732/ajb.1700165>)
140. Soto W, Punke EB, Nishiguchi MK. 2012 Evolutionary perspectives in a mutualism of sepiolid squid and bioluminescent bacteria: combined usage of microbial experimental evolution and temporal population genetics. *Evolution* **66**, 1308-1321. (DOI: 10.1111/j.1558-5646.2011.01547.x)
141. Koch EJ, Miyashiro T, McFall-Ngai MJ, Ruby EG. 2014 Features governing symbiont persistence in the squid–vibrio association. *Mol. Ecol.* **23**, 1624-1634. (<https://doi.org/10.1111/mec.12474>)
142. Scheuring I, Yu DW. 2012 How to assemble a beneficial microbiome in three easy steps. *Ecol. Lett.* **15**, 1300-1307. (<https://doi.org/10.1111/j.1461-0248.2012.01853.x>)
143. Ranger CM, Biedermann PH, Phuntumart V, Beligala GU, Ghosh S, Palmquist DE, Mueller R, Barnett J, Schultz PB, Reding ME, Benz JP. 2018 Symbiont selection via alcohol benefits fungus farming by ambrosia beetles. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 4447-4452. (<https://doi.org/10.1073/pnas.1716852115>)
144. Chomicki G, Renner SS. 2016 Obligate plant farming by a specialized ant. *Nat. Plants* **2**, 16181. (<https://doi.org/10.1038/nplants.2016.181>)
145. Chomicki G, Renner SS. 2019 Farming by ants remodels nutrient uptake in epiphytes. *New Phytol.* **223**, 2011–2023. (<https://doi.org/10.1111/nph.15855>)
146. Chomicki, G., Kadereit, G., Renner, S.S. and Kiers, E.T., 2020. Tradeoffs in the evolution of plant farming by ants. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 2535-2543. (<https://doi.org/10.1073/pnas.1919611117>)
147. Schluter J, Foster KR. 2012 The evolution of mutualism in gut microbiota via host epithelial selection. *PLoS Biol.* **10**, e1001424. (<https://doi.org/10.1371/journal.pbio.1001424>)
148. West SA, Kiers ET, Pen I, Denison RF. 2002 Sanctions and mutualism stability: when should less beneficial mutualists be tolerated? *J. Evol. Biol.* **15**, 830-837. (<https://doi.org/10.1046/j.1420-9101.2002.00441.x>)
149. Palmer TM, Doak DF, Stanton ML, Bronstein JL, Kiers ET, Young TP, Goheen JR, Pringle RM. 2010 Synergy of multiple partners, including freeloaders, increases host fitness in a multispecies mutualism. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 17234-17239. (<https://doi.org/10.1073/pnas.1006872107>)
150. Steele MI, Kwong WK, Whiteley M, Moran NA. 2017. Diversification of type VI secretion system toxins reveals ancient antagonism among bee gut microbes. *MBio* **8**, e01630-17. (DOI: 10.1128/mBio.01630-17)
151. Braendle C, Miura T, Bickel R, Shingleton AW, Kambhampati S, Stern DL. 2003. Developmental origin and evolution of bacteriocytes in the aphid–*Buchnera* symbiosis. *PLoS Biol.* **1**, 70-76. (DOI: 10.1371/journal.pbio.0000021)

152. Monnin D, Jackson R, Kiers ET, Bunker M, Ellers J, Henry LM. 2020. Parallel evolution in the integration of a co-obligate aphid symbiosis. *Curr. Biol.* (<https://doi.org/10.1016/j.cub.2020.03.011>)
153. Salem H, Bauer E, Kirsch R, Berasategui A, Cripps M, Weiss B, Koga R, Fukumori K, Vogel H, Fukatsu T, Kaltenpoth M. 2017. Drastic genome reduction in an herbivore's pectinolytic symbiont. *Cell* **171**, 1520-1531. (<https://doi.org/10.1016/j.cell.2017.10.029>)
154. Yang Y, Sun J, Sun Y, Kwan YH, Wong WC, Zhang Y, Xu T, Feng D, Zhang Y, Qiu JW, Qian PY. 2020. Genomic, transcriptomic, and proteomic insights into the symbiosis of deep-sea tubeworm holobionts. *ISME J.* **14**, 135-150. (<https://doi.org/10.1038/s41396-019-0520-y>)
155. Gruber-Vodicka HR, Leisch N, Kleiner M, Hinzke T, Liebeke M, McFall-Ngai M, Hadfield MG, Dubilier N. 2019. Two intracellular and cell type-specific bacterial symbionts in the placozoan *Trichoplax* H2. *Nat. Microbiol.* **4**, 1465-1474. (<https://doi.org/10.1038/s41564-019-0475-9>)
156. Karamipour N, Fathipour Y, Mehrabadi M. 2016. Gammaproteobacteria as essential primary symbionts in the striped shield bug, *Graphosoma lineatum* (Hemiptera: Pentatomidae). *Sci. Rep.* **6**, 33168. (DOI: 10.1038/srep33168)

Figure captions

Figure 1. Diversity of host compartmentalization in symbioses across the tree of life. (a-d) Strict symbiont compartmentalization in which symbionts are fully enclosed by well-defined, permanent compartment boundary typically with one genotype per compartment. (a) Nested bacteriocyte in cicadas. Green shows *Sulcia* symbionts; red shows *Hodgkinia* symbionts; magenta shows cicada nuclei. (b) Z-projection of confocal sections depicting the chlorophyll autofluorescence of *Chlorella* endosymbionts within one *Paramecium bursaria* cell. With colours representing the intensity of fluorescence and therefore the position of the *Chlorella* in the Z-plane. (c) *Lotus* (legume) nodules harbour thousands of *Rhizobia* bacteria. (d) Hawaiian bobtail squid harbours luminescent bacteria in individual crypts (e-h) Fluid symbiont compartmentalization in which symbionts are enclosed by less or non-discrete or permanent boundaries. (e) Vertebrate skin harbours a diverse microbiome in more open compartments. (f) Fluorescence micrograph of two bacterial gut symbionts (*Leptomonas pyrrhocoris* (green) and *Coriobacterium glomerans* (red) in a faecal droplet from the firebug *Dysdercus fasciatus*. Counterstaining of DNA with DAPI (blue). (g) Representative fluorescence in situ hybridization (FISH) image of a distal colon section in a mouse fed with a conventional diet. The picture shows all bacteria stained with EUB 338 I-III (green), the eukaryotic cells stained with EUK-516 (pink) and DNA stained with DAPI (blue). (h) In fungus-farming ants, open crypts occur below the ant's thorax, allowing the development of nested population of antibiotic-producing bacteria. (i) Partial compartmentalization with only the nutrient exchange structure compartmentalised. Arbuscular of an arbuscular mycorrhizal fungus. (j-k) Compartmentalization in mutualisms with non-microbial partners. In the fig/fig-wasp and yucca moth mutualism (j) and in the *Glochidion/Epicephala* moth mutualism (k), each inflorescence (fig) or flower (*Glochidion*) acts as a compartment that can be shed if the moth consumes too many seeds and does not pollinate. (l) Absence of compartmentalization. CLSM image of deep-sea mussel *Bathymodiolus azoricus*

whole gill cross-section based on direct gene FISH in which up to 16 symbionts can coexist. Genetic marker (red: Alexa Fluor 647), sulfur-oxidizing symbionts (SOX) 16S rRNA (Atto488; green). Photo credit: (a) James Van Leuven & John McCutcheon; (b) Ewan Minter; (c-e, i, j) Wikipedia; (f) Hassan Salem; (g) Alessandra Riva; (h) Hongjie Li; (k) Shixiao Luo; (l) Miguel Angel;.

Table 1. Compartments are divided into four main types according to their how they enclose their partners (Table 1, Figure 1). These are: (i) strict compartmentalization, where symbionts, often single genotypes, are fully enveloped by well-defined, (semi) permanent boundaries, (ii) fluid compartmentalization, where the boundaries of the compartment are less discrete or permanent, (iii) partial compartmentalization, where only part of the symbiont – such as the nutrient exchange structure - is compartmentalized (iv) compartmentalization of non-microbial partners, whereby the enclosed mutualist is itself a large macro-organism. (?) = tests of these mechanisms are not yet known.

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Host	Symbiont	Name of compartment	Containment to prevent spread within host tissue	Control reproduction	Mediate discrimination	Control resource allocation	Reduce within host conflict	References
1. Host-microbe mutualisms – Strict compartmentalization								
Cicadas, Mealy bugs	<i>Sulcia</i> , <i>Hodgkinia</i> , Yeast-like fungi	Bacteriocytes within Bacteriomes	Yes	Yes	?	?	Yes	[32, 47]
Aphids	<i>Buchnera</i> , <i>Serratia</i>	Bacteriocyte	Yes	Yes	No (?)	?	Yes	[151, 152]
<i>Cassida rubiginosa</i> leaf beetle	<i>Stammera</i>	Extracellular bacterium housed in specialized organs connected to the foregut.	Yes	Yes	?	?	No(?)	[153]
Squid	<i>Vibrio</i> bacteria	Crypts	Yes	?	Yes	?	Yes	[89, 98]
Legumes, <i>Parasponia</i>	<i>Rhizobia</i>	Symbiosomes within nodules (legumes) Intracellular fixation threads in nodule cells (<i>Parasponia</i>)	Yes	Yes, in some legume lineages	Yes	Yes	Yes	[72, 102, 126]
Coral	<i>Symbionidium</i>	Symbiosome (host vacuole)	Yes	Yes	?	Yes	No	[34, 39]
<i>Paracatenula</i> flatworm	<i>Alphaproteobacteria</i>	Trophosome	Yes	Yes	No (?)	Yes	No (?)	[44, 154]
<i>Trichoplax</i> sp. H2	<i>Grellia incantans</i> and <i>Ruthmannia eludens</i>	Rough endoplasmic reticulum of the host's internal fibre cells [<i>G. incantans</i>]; ventral epithelial cells [<i>Ruthmannia eludens</i>]	Probably	?	?	?	Probably	[155]
2. Host-microbe mutualisms – Fluid compartmentalization								
Shield bug	<i>Gammaproteobacteria</i>	Symbiont capsules, jelly secretions, Faecal droplets	No	Yes	No (?)	No (?)	No (?)	[24, 156]
Humans	Skin microbiome	Defined niches, such as hair follicles	Yes	?	No (?)	No (?)	No (?)	[58]
Bean bug	<i>Burkholderia</i>	Gut crypts	Yes (?)	No	No (?)	?	Yes, by reducing physical contact	[133]
Vertebrates	Gut microbiome	Lamina propria, Peyer's patches	Yes	?	No (?)	?	?	[54]
Honey Bee, Termite	Gut microbiome	Midgut, Ileum, Rectum	Yes	?	No (?)	?	?	[55, 56]

Attine ants	<i>Pseudonocardia bacteria</i>	Specialized cuticular structures, including crypts and tubercles	No	Yes	No (?)	No (?)	Yes (?)	[64, 65]
3. Host-microbe mutualisms – Partial compartmentalization								
Plants	Arbuscular mycorrhizal fungi	Root cell containing arbuscule	No	No	Yes	Yes	Yes	[107]
4. Compartmentalization in non-microbial mutualisms								
Yucca	Yucca-moth	Entire flower	No	Yes	Yes	-	?	[119]
Fig	Fig-wasp	Entire inflorescence (fig)	No	Yes	Yes	-	?	[[122, 124]
Glochidion	<i>Epicephala</i> moths	Entire flower	No	Yes	Yes	-	?	[118]

